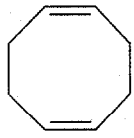
Existing published and unpublished data were collected and scientifically evaluated to determine the best possible study or studies to be summarized for each required endpoint. In the spirit of this voluntary program, other data of equal or lesser quality are not summarized, but are listed as related references at the end of each appropriate section, with a statement to reflect the reason why these studies were not summarized.

1.0 Substance Information

CAS Number: 111-78-4

Chemical Name: 1,5-Cyclooctadiene

Structural Formula:



Other Names: COD
1,5-COD
Cycloocta-1,5-diene
NMT-NOVA-11
NOVA-11

Exposure Limits: 10 ppm, 8- and 12-hour TWA: DuPont Acceptable Exposure Limit (AEL)

2.0 Physical/Chemical Properties

2.1 Melting Point

Value:	-70 to -69°C
Decomposition:	No Data
Sublimation:	No Data
Pressure:	No Data
Method:	No Data
GLP:	Unknown
Reference:	Weast, R. C. (ed.) (1979). Handbook of Chemistry and Physics, 60th ed., p. C-266, CRC Press Inc., Boca Raton, FL (HSDB/5549).
Reliability:	Not assignable because limited study information was available.

Additional References for Melting Point:

Brown, V. K. H. and C. G. Hunter (1968). Br. J. Ind. Med., 25:75-76.

DuPont Co. (2000). Material Safety Data Sheet 6015CR (January 26).

Lide, D. R. (ed.) (2001). CRC Handbook of Chemistry and Physics, 82nd ed., CRC Press, Boca Raton, FL.

2.2 Boiling Point

Value:	150.8°C
Decomposition:	No Data
Pressure:	757 mm Hg
Method:	No Data
GLP:	Unknown
Reference:	Weast, R. C. (ed.) (1979). Handbook of Chemistry and Physics, 60th ed., p. C-266, CRC Press Inc., Boca Raton, FL (HSDB/5549).
Reliability:	Not assignable because limited study information was available.

Additional References for Boiling Point:

Brown, V. K. H. and C. G. Hunter (1968). Br. J. Ind. Med., 25:75-76.

DuPont Co. (2000). Material Safety Data Sheet 6015CR (January 26).

Lide, D. R. (ed.) (2001). CRC Handbook of Chemistry and Physics, 82nd ed., CRC Press, Boca Raton, FL.

2.3 Density

Value:	Specific Gravity = 0.8818
Temperature:	25/4°C
Method:	No Data
GLP:	Unknown
Results:	No additional data.
Reference:	Weast, R. C. (ed.) (1979). Handbook of Chemistry and Physics, 60th ed., p. C-266, CRC Press Inc., Boca Raton, FL (HSDB/5549).
Reliability:	Not assignable because limited study information was available.

Additional References for Density:

Brown, V. K. H. and C. G. Hunter (1968). Br. J. Ind. Med., 25:75-76.

DuPont Co. (2000). Material Safety Data Sheet 6015CR (January 26).

Lide, D. R. (ed.) (2001). CRC Handbook of Chemistry and Physics, 82nd ed.,

CRC Press, Boca Raton, FL.

NFPA (National Fire Protection Association) (1978). Fire Protection Guide on Hazardous Materials, 7th ed., p. 325M-60, National Fire Protection Association, Boston, MA (HSDB/5549).

2.4 Vapor Pressure

Value:	6.8 mm Hg
Temperature:	25°C
Decomposition:	No Data
Method:	No Data
GLP:	Unknown
Reference:	DuPont Co. (2000). Material Safety Data Sheet 6015CR (January 26).
Reliability:	Not assignable because limited study information was available.

Additional Reference for Vapor Pressure:

Hawley, G. G. (1977). The Condensed Chemical Dictionary, 9th ed., p. 249, Van Nostrand Reinhold Co., New York (HSDB/5549).

2.5 Partition Coefficient (log Kow)

Value:	3.16
Temperature:	No Data
Method:	The procedures used in the test were based on the recommendations of the following guideline: OECD Guideline 107.
GLP:	Unknown
Reference:	Eadsforth, C. V. and P. Moser (1983). Chemosphere, 12(11):1459-1475.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Partition Coefficient (log Kow): None Found.

2.6 Water Solubility

Value:	64.11 mg/L
Temperature:	25°C
pH/pKa:	No Data
Method:	Estimated from a measured log Kow of 3.16. The estimation is from (Meylan and Howard, 1994a;1994b) and described in

Meylan et al., 1996 and in a document prepared for the U.S. Environmental Protection Agency (OPPT): Upgrade of PCGEMS Water Solubility Estimation Method (May 1994).

GLP: Not Applicable

Reference: WSKOW v.1.40 in EpiWin v3.05 (SRC Database).

Meylan, W. M. and P. H. Howard (1994a). Upgrade of PCGEMS Water Solubility Estimation Method (May 1994 Draft); prepared for Robert S. Boethling, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC; prepared by Syracuse Research Corporation, Environmental Science Center, Syracuse, NY 13210.

Meylan, W. M. and P. H. Howard (1994b). Validation of Water Solubility Estimation Methods Using Log Kow for Application in PCGEMS & EPI (Sept 1994, Final Report); prepared for Robert S. Boethling, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC; prepared by Syracuse Research Corporation, Environmental Science Center, Syracuse, NY 13210.

The following journal article describes the estimation methodology:

Meylan, W. M. et al. (1996). Environ. Toxicol. Chem., 15:100-106.

Reliability: Estimated value based on accepted model.

Additional Reference for Water Solubility:

DuPont Co. (2000). Material Safety Data Sheet 6015CR (January 26).

Weast, R. C. (ed.) (1979). Handbook of Chemistry and Physics, 60th ed., p. C-266, CRC Press Inc., Boca Raton, FL (HSDB/5549).

2.7 Flash Point

Value: 35°C

Method: No Data

GLP: Unknown

Reference: NFPA (1978). Fire Protection Guide on Hazardous Materials, 7th ed., p. 325M-60, National Fire Protection Association, Boston, MA (HSDB/5549).

Reliability: Not assignable because limited study information was

available.

Additional Reference for Flash Point:

DuPont Co. (2000). Material Safety Data Sheet 6015CR (January 26).

2.8 Flammability

Results:	Flammable liquid (Autoignition Temperature = 223°C)
Method:	No Data
GLP:	Unknown
Reference:	DuPont Co. (2000). Material Safety Data Sheet 6015CR (January 26).
Reliability:	Not assignable because limited study information was available.

Additional References for Flammability: None Found.

3.0 Environmental Fate

3.1 Photodegradation

Concentration:	No Data
Temperature:	25°C
Direct Photolysis:	No Data
Indirect Photolysis:	If released to the atmosphere, 1,5-cyclooctadiene (COD) is expected to photodegrade rapidly. The estimated rate constant for the reaction of COD vapor with photochemically generated hydroxyl radicals in the atmosphere has been estimated to be 1.173953×10^{-10} cm^3/molecule-sec at 25°C. This value corresponds to a reaction half-life of 0.137 days, assuming an ambient hydroxyl radical concentration of 0.5×10^6 molecules/cm^3. The estimated rate constant for the reaction with ozone in the atmosphere has been estimated to be 40.00×10^{-17} cm^3/molecule-sec at 25°C. This value corresponds to a reaction half-life of 0.029 days, assuming an ambient ozone concentration of 7×10^{11} molecules/cm^3.
Breakdown Products:	No Data
Method:	Calculated by AOP Computer Program, Vers. 1.90, Syracuse Research Corporation. The AOP Program is described in Meylan and Howard, 1993.
GLP:	Not Applicable
Reference:	Meylan, W. M. and P. H. Howard (1993). Chemosphere, 26:2293-2299.
Reliability:	Estimated value based on accepted model.

Additional References for Photodegradation: None Found.

3.2 Stability in Water

Concentration:	Not Applicable
Half-life:	Estimated half-life values for a model river and model lake are 1.084 and 99.04 hours, respectively.
% Hydrolyzed:	Not Applicable
Method:	The Henry's Law constant for COD is estimated to be 0.01101 atm-m^3/mole (Henry v3.10 Program, VP/Wsol estimate using EPA values in SRC EPIWIN v3.05) from its estimated vapor pressure (4.96 mm Hg) and estimated water solubility (64.1 mg/L). Based on this Henry's Law constant, the estimated volatilization half-life from a model river (1 m deep, flowing 1 m/s, wind velocity of 3 m/sec) is approximately 1.084 hours. The estimated volatilization half-life from a model lake (1 m deep, flowing 0.05 m/sec, wind velocity of 0.5 m/sec) is approximately 99.04 hours (EPIWIN v3.05).
GLP:	Not Applicable
Reference:	Syracuse Research Corporation EPIWIN v3.05.
Reliability:	Estimated value based on accepted model.

Additional References for Stability in Water: None Found.

3.3 Transport (Fugacity)

Media:	Air, Water, Soil, and Sediments	
Distributions:	Air:	0.613%
	Water:	59.7%
	Soil:	38.4%
	Sediments:	1.35%
Half-life:	Air:	0.568 hours
	Water:	360 hours
	Soil:	720 hours
	Sediments:	3240 hours
Adsorption Coefficient:	Not Applicable	
Desorption:	Not Applicable	
Volatility:	Not Applicable	
Method:	Calculated according to Mackay, Level III, Syracuse Research Corporation EPIWIN v3.05. Emissions (1000 kg/hr) to air, water, and soil compartments using standard EPA model defaults.	

	Data Used: Molecular Weight: 108.18 Henry's Law Constant: 0.01101 atm-m^3/mole (HenryWin Program) Vapor Pressure: 4.96 mm Hg Log Kow: 3.16 (measured) Soil Koc: 593 (calculated by model)
GLP:	Not Applicable
Reference:	Syracuse Research Corporation EPIWIN v3.05 contains a Level III fugacity model. The methodology and programming approach were developed by Dr. Donald MacKay and coworkers and are detailed in: Mackay, D. (1991). Multimedia Environmental Models: The Fugacity Approach, pp. 67-183, Lewis Publishers, CRC Press. Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1618-1626. Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1627-1637.
Reliability:	Estimated value based on accepted model.

Additional References for Transport (Fugacity): None Found.

3.4 Biodegradation

Value:	Primary biodegradation is estimated to occur in days to weeks. Ultimate biodegradation is estimated to occur in weeks.
Breakdown Products:	Unknown for primary biodegradation. Carbon dioxide and water for ultimate biodegradation.
Method:	BIOWIN v4.00
GLP:	Not Applicable
Reference:	The Biodegradation Probability Program (BIOWIN for MS-Windows, v.4) as reviewed by Boethling et al., 1994; Howard et al., 1987; Howard et al., 1992; and Tunkel et al., 2000, used as part of the EpiWin 3.05 (7/30/02) Suite (Syracuse Research Corporation). Howard, P. H. et al. (1992). Environ. Toxicol. Chem., 11:593-603. Howard, P. H. et al. (1987). Environ. Toxicol. Chem., 6:1-10.

Boethling, R. S. et al. (1994). Environ. Sci. Technol., 28:459-65.

Tunkel, J. et al. (2000). Environ. Toxicol. Chem., 19(10):2478–2485.

Reliability: Estimated value based on accepted model.

Additional References for Biodegradation:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Bridie, A. L. et al. (1979). Water Res., 13(7):627-630.

Shell Oil Co. (1982). TSCA Fiche OTS0206201.

Shell Oil Co. (1974). TSCA Fiche OTS0206200.

Shell Oil Co. (1982). TSCA Fiche OTS0206201.

3.5 Bioconcentration

Value:	Estimated BCF = 54.1 (log BCF = 1.733)
Method:	Calculated by BCFWIN Computer Program, Vers. 2.14, Syracuse Research Corporation (based on reference below). The log Kow value used to make the BCF estimate was 3.16.
GLP:	Not Applicable
Reference:	The estimation methodology used by BCFWIN is described in the following document prepared for the U.S. Environmental Protection Agency (OPPT): "Improved Method for Estimating Bioconcentration Factor (BCF) from Octanol-Water Partition Coefficient," SRC TR-97-006 (2nd Update), July 22, 1997; prepared for Robert S. Boethling, EPA-OPPT, Washington, DC; Contract No. 68-D5-00012; prepared by William M. Meylan, Philip H. Howard, Dallas Aronson, Heather Printup, and Sybil Gouchie; Syracuse Research Corp., Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212.
Reliability:	Estimated value based on accepted model.

Additional References for Bioconcentration: None Found.

4.0 Ecotoxicity

4.1 Acute Toxicity to Fish

Type:	**48- and 96-hour LC_{50}**
Species:	*Salmo gairdneri* (Rainbow trout)
Value:	30-38 mg/L
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. 1,5-Cyclooctadiene was dissolved in acetone to give a 100 mg/mL solution. Volumes of this solution were dispensed into a series of 10 L all-glass aquaria containing dechlorinated mains-water (total hardness 260 mg/L; pH 7.5) to give a series of logarithmically spaced concentrations (0, 27.6, 30.0, 38.1 mg/L). Acetone was added to the control aquarium to give a concentration of 1000 mg/L. The pH of the aquarium contents was 7.5 and the temperature was maintained at 15°C. Continuous gentle aeration was applied to maintain the oxygen concentration of the aquarium contents above 7 mg/L. Five fish, weighing between 1 and 2 g, were placed into each aquarium and the mortality was recorded during the subsequent 96 hours.
GLP:	No
Test Substance:	1,5-Cyclooctadiene, purity ≥ 98%
Results:	Mortality at 96 hours was 0/5, 0/5, 1/5, and 5/5 at 0, 27.6, 30.0, and 38.1 mg/L. No additional data was reported.
Reference:	Shell Oil Company (1977). Unpublished Data, Group Research Report GRR – TLGR.0139.77, "Acute Toxicity of 1,5-Cyclo-octadiene to Rainbow Trout (*Salmo Gairdneri*) and *Daphnia Magna*" (TSCA Fiche OTS0537406).
Reliability:	Medium because a suboptimal study design was used (nominal test concentrations).

Type:	**96-hour LC_{50}**
Species:	Fish
Value:	6.5 mg/L
Method:	Modeled, using log Kow of 3.16.
GLP:	Not Applicable
Test Substance:	1,5-Cyclooctadiene
Results:	The estimated value of 6.5 mg/L is consistent with the reported test data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S.

Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).

Reliability: Estimated value based on accepted model.

Additional Reference for Acute Toxicity to Fish:

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Bridie, A. L. et al. (1979). Water Res., 13(7):623-626.

4.2 Acute Toxicity to Invertebrates

Type:	**24-hour LC_{50}**
Species:	*Daphnia magna*
Value:	0.9 mg/L (95% confidence limits, 0.6-1.4 mg/L)
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Volumes of acetone dilutions of the solution of 1,5-cyclooctadiene were dispensed into a series of 250 mL glass beakers containing 200 mL of deionized water with salts added to ISO (International Standards Organisation (1977); proposed method for the testing of chemicals and effluents to *Daphnia magna*, ISO/TC147/SC5/WG2) specifications (total hardness 100 mg/L, pH 8.0 ± 0.2) to give a series of logarithmically spaced concentrations (0, 0.37, 0.56, 0.85, 1.29, 1.96, 3.00, and 4.58 mg/L). Acetone was added to the control beakers to give a concentration of 300 mg/L. The contents of the beakers were maintained at 20 ± 2°C, and had a pH of 8.0. Ten *Daphnia* (aged 0-24 hours) were placed in each beaker. After 24 hours, the number of reely swimming *Daphnia* were counted, from which the percentage immobilization in each beaker was deduced.
GLP:	No
Test Substance:	1,5-Cyclooctadiene, purity ≥ 98%
Results:	Immobilization after 24 hours of exposure was 0/10, 3/10, 3/10, 4/10, 5/10, 8/10, 8/10, and 10/10 at 0, 0.37, 0.56, 0.85, 1.29, 1.96, 3.00, and 4.58 mg/L. No additional data was reported.
Reference:	Shell Oil Company (1977). Unpublished Data, Group

	Research Report GRR – TLGR.0139.77, "Acute Toxicity of 1,5-Cyclo-octadiene to Rainbow Trout (*Salmo Gairdneri*) and *Daphnia Magna*" (TSCA Fiche OTS0537406).
Reliability:	Medium because a suboptimal study design was used (nominal test concentrations).

Type:	**48-hour EC_{50}**
Species:	*Daphnia*
Value:	7.6 mg/L
Method:	Modeled, using log Kow of 3.16.
GLP:	Not Applicable
Test Substance:	1,5-Cyclooctadiene
Results:	The estimated value of 7.6 mg/L is consistent with the reported test data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional Reference for Acute Toxicity to Invertebrates: None Found.

4.3 Acute Toxicity to Aquatic Plants

Type:	**96-hour EC_{50}**
Species:	Green algae
Value:	5.0 mg/L
Method:	Modeled, using a log Kow of 3.16.
GLP:	Not Applicable
Test Substance:	1,5-Cyclooctadiene
Results:	The 96-hour ChV was 1 mg/L.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC; prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Aquatic Plants: None Found.

5.0 Mammalian Toxicity

5.1 Acute Toxicity

Type:	**Oral LD_{50}**
Species/Strain:	Rats/Strain not defined
Value:	2.7 mL/kg
Method:	No specific test guideline or information regarding method was reported.
GLP:	Unknown
Test Substance:	1,5-Cyclooctadiene, purity not specified
Results:	No additional data.
Reference:	Shell Oil Co. (1975). Shell Industrie Chemicalien gids, Shell Nederland Chemie, Afd. Industrie-chemicalien, Wassenaarseweg 80, 's-Gravenhage, Nederland, January 1 (cited in Verschueren, K. (1983). Handbook of Environmental Data of Organic Chemicals, 2nd ed., p. 426, Van Nostrand Reinhold Co., New York, NY).
Reliability:	Not assignable because limited study information was available.

Additional References for Acute Oral Toxicity:

Data from this additional source were not summarized because the study design was not adequate.

Gerarde, H. W. (1963). Arch. Environ. Health, 6(3):329-341.

Type:	**Inhalation ALC**
Species/Strain:	Male rats/ Crl:CD®BR
Exposure Time:	4 hours
Value:	2700 ppm
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Three groups of 6 male Crl:CD®BR rats each were exposed nose-only for a single, 4-hour period to vapors of the test substance in air at chamber vapor concentrations of 1400, 2700, or 4300 ppm. The rats were approximately 8 weeks old and ranged in weight from 268-292 grams at the time of exposure. Chamber atmospheres were generated by vaporizing the test substance in a heated nitrogen stream. The test substance was metered into a heated Instaterm flask with a syringe

infusion pump. The resulting vapor was carried by a metered stream of nitrogen into a tube where houseline air was added to dilute the vapor. The resulting mixture then flowed into the exposure chamber. A dispersion plate located at the chamber inlet helped to distribute the vapor evenly throughout the chamber. Chamber concentrations were controlled by varying the test substance feed rate. Atmospheric concentrations were measured at approximately 15-minute intervals by gas chromatography. The atmospheric concentration of the test substance was determined by comparing the detector response of the chamber air samples with gas standards that were prepared prior to each exposure.

Chamber airflow was set at the beginning of each exposure to achieve the desired chamber atmospheric concentration. Total chamber airflow was monitored continually, and the airflow was recorded at the beginning of each exposure. Chamber temperature was targeted at 23 ± 2°C, measured continually, and recorded 3 times during each exposure. The chamber relative humidity was targeted at 50 ± 10%, and was measured 3 times during each exposure. Chamber oxygen concentration was targeted to at least 19% and was measured 3 times during exposure.

Rats were observed for mortality and response to stimuli during exposure, and clinical signs of toxicity and mortality immediately following exposure. During a 14-day post-exposure period, rats from all groups were observed each day for mortality, and were weighed and observed for clinical signs of toxicity daily (weekends excluded unless warranted by the health status of the rats).

Descriptive statistics (e.g., mean, standard deviation, etc.) were used, where appropriate, to summarize experimental data. Regression analyses, probit analyses, or other techniques may have been used to evaluate analytical data. Ordinarily, differences were judged statistically significant at $p \leq 0.05$.

GLP: Yes

Test Substance: 1,5-Cyclooctadiene, purity > 99%

Results: Mortality was 0/6, 1/6, and 4/6 at 1400, 2700, and 4300 ppm, respectively. Deaths observed at 4300 ppm occurred during exposure, and the death observed at 2700 ppm occurred overnight. By 3 hours into the exposure, all rats failed to respond to external sound stimuli. Upon

removal from the exposure chamber, the 2 surviving rats at 4300 ppm and 4 rats at 2700 ppm were immobile. Three rats at 1400 ppm exhibited ataxia. Other clinical signs of toxicity observed immediately following exposure included ocular discharge, lethargy, and irregular respiration. Except for ocular discharge, these clinical signs were not observed the day after exposure or during the remainder of the 14-day observation period. Ocular discharge, ruffled fur, or stained perineum were observed up to 4 days following exposure. No clinical signs were observed throughout the remaining observation period. Most surviving rats experienced slight to severe body weight losses (4-14% of initial body weight) the day following exposure. These rats subsequently began gaining weight and did not experience weight loss throughout the remainder of the recovery period.

Prior to initiation of the test exposures, chamber distribution of vapor was evaluated. No statistical differences were observed with the Student's t-test ($\alpha = 0.05$), when samples taken at 3 locations in the face plate were compared to 3 samples taken from the reference sampling port. The test substance, therefore, was considered to be homogeneously distributed throughout the exposure chamber. Chamber temperature ranged from 23-26°C, chamber relative humidity ranged from 42-45%, chamber airflow was 25 L/min, and the oxygen concentration was 19-20%.

On an acute inhalation basis, this material is considered slightly toxic.

Reference: DuPont Co. (1996). Unpublished Data, Haskell Laboratory Report No. 177-96, "Inhalation Approximate Lethal Concentration (ALC) in Rats" (April 3).

Reliability: High because a scientifically defensible or guideline method was used.

Additional References for Acute Inhalation Toxicity:

Data from this additional source supports the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

DuPont Co. (1966). Unpublished Data, Haskell Laboratory No. 126-66, "Acute Inhalation Toxicity" (August 8).

Data from this additional source were not summarized because the test substance was a mixture or otherwise inappropriate.

DuPont Co. (1966). Unpublished Data, Haskell Laboratory Report No. 114-66, "Acute Inhalation Toxicity" (August 1).

Type:	**Dermal LD_{50}**
Species/Strain:	Male and female rats/CD
Exposure Time:	24 hours
Value:	> 4 mL/kg (> 3520 mg/kg)
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Two rats of each sex (aged 12-13 weeks) were used at each dose level (2, 3, or 4 mL/kg). The test substance was placed onto the shorn dorso-lumbar skin, and bandaged to contact the skin using an impermeable dressing of aluminum foil and waterproof plaster. The rats were housed individually over the 24-hour exposure period, during which time they were deprived of food, but allowed water *ad libitum*. After 24 hours, the dressings were removed and the exposed area was washed with a tepid dilute detergent solution. The rats were then housed 3/cage, genders separate, and observed for signs of intoxication during the following 9 days (The method used was further described in Noakes, D. W. and D. M. Sanderson (1969). Br. J. Ind. Med., 26:59-64).
GLP:	Unknown
Test Substance:	1,5-Cyclooctadiene, purity not specified
Results:	The rats squealed when the test substance was first placed on their backs, however, when the occlusive dressings were removed no signs of any skin irritation were observed. No mortality was observed at any dose level.
Reference:	Shell Research Limited (1976). Unpublished Data, Group Research Report TLGR.0025.76, "Toxicity of Fine Chemicals: Acute Percutaneous Toxicity and Skin Corrosivity of 1,5-Cyclooctadiene and 1,5,9-Cyclododecatriene" (May).
Reliability:	Medium because a suboptimal study design was used.

Additional Reference for Acute Dermal Toxicity: None Found.

Type:	**Dermal Irritation**
Species/Strain:	Male and female rabbits/ New Zealand White Male and female guinea pigs/"P" Female mice/CAH
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the

study.

The tests were of 2 types, those in which the skin was exposed to the test substance inside an impermeable covering, and those in which the skin was treated with free access to the atmosphere.

Two male and 2 female albino rabbits were used. The dorsal hair between the shoulders and the hindquarters was closely shorn. On the 1st, 2nd, and 3rd days of the test, the rabbits were immobilized for periods of 6 hours in a special holding device. Patches of lint were cut, and 1 mL of the test substance was applied to each. Two patches were laid on each rabbit's back and covered with a sheet of thin polythene; these were bandaged into position by means of an open-weave bandage. A visual assessment of the degree of erythema and edema, ranging from 0 (no erythema or no edema, respectively) to 4 (beet redness or severe edema, respectively). Notes were made of any other gross changes. Seven days after the 1st application, the final visual assessment was made, and specimens of the rabbits' skins were taken for histopathological examination.

Rabbits (1/sex) and guinea pigs (5/sex) were shorn as described above on the Monday of each week of the test. Daily, 5 days/week for 4.5 weeks, 1 mL (rabbits) or 0.5 mL (guinea pigs) of the test substance was dropped onto the shorn area near the midline. A daily visual assessment was made of the gross skin damage. On the day after the 23rd application of test substance, the skin was removed for histopathological examination.

Two female hairless mice were used. Undiluted test substance was painted onto the ventral skin on 12 consecutive days by means of fine paint brushes. A visual assessment of the skin damage was made daily.

GLP: No

Test Substance: 1,5-Cyclooctadiene, purity 98%

Results: In the covered test in rabbits, the irritation caused by the test substance was very severe. Epidermal sloughing was particularly apparent after the application of 1,5-cyclooctadiene. The use of sulphan blue showed the skin to be grossly injured. Histopathological examination of skin taken from the rabbits post-mortem revealed necrosis of the epidermis, and ulceration and marked inflammation of the dermis.

	In the uncovered application tests in rabbits, guinea pigs, and "hairless" mice, 1,5-cyclooctadiene produced an immediate erythematous reaction after only 1 application. A severe acute contact dermatitis followed with epidermal sloughing. Hair growth was suppressed around the shorn sites of application in the rabbits and guinea-pigs. Because of the severity and nature of the reaction, a group of guinea pigs was treated as above, but not killed until 3 weeks after the last application of the test substance. Histological examination of the skins from these animals showed that epidermal thickening and considerable acanthosis were still present.
Reference:	Brown, V. K. H. and C. G. Hunter (1968). Br. J. Ind. Med., 25:75-76. Brown, V. K. H. et al. (1967). Ann. Occup. Hyg., 10:123-126. Hunter, G. G. et al. (1966). Br. J. Ind. Med., 23:137-141.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional Reference for Dermal Irritation:

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Shell Research Limited (1976). Unpublished Data, Group Research Report TLGR.0025.76, "Toxicity of Fine Chemicals: Acute Percutaneous Toxicity and Skin Corrosivity of 1,5-Cyclooctadiene and 1,5,9-Cyclododecatriene" (May).

Type:	**Dermal Sensitization**
Species/Strain:	Guinea pigs/Strain not specified
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Tests were carried out by subcutaneous injection or by applying a 0.1% w/v solution in light liquid paraffin to the shorn skin on the backs of guinea pigs on 3 days in each of 3 successive weeks. The animals then received no treatment for 10 days, and a challenge dose of the same solution on the right flank and of solvent on the left flank on the 11th day. Following the challenge, the guinea pigs were examined at 1,

	24, and 48 hours for signs of a sensitization reaction (i.e., intense redness, wealing, or both). Subjective assessments were made of the reaction.
GLP:	No
Test Substance:	1,5-Cyclooctadiene, purity 98%
Results:	1,5-Cyclooctadiene was a potent skin sensitizer in guinea pigs when tested as 0.1% w/v solution in light liquid paraffin. In the topical test at 24 and 48 hours 10/10 guinea pigs showed positive sensitive reactions. In the intradermal test at 24 and 48 hours 9/10 guinea pigs showed positive sensitive reactions.
Reference:	Brown, V. K. H. and C. G. Hunter (1968). Br. J. Ind. Med., 25:75-76. Brown, V. K. H. et al. (1967). Ann. Occup. Hyg., 10:123-126. Hunter, G. G. et al. (1966). Brit. J. Industr. Med., 23:137-141.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Dermal Sensitization: None Found.

Type:	**Eye Irritation**
Species/Strain:	Species not defined/Strain not defined
Method:	The method used was that described in the United States Federal Register (1964) and the assessment of irritancy was based on the recommendations of the United States Department of Health, Education, and Welfare.
GLP:	No
Test Substance:	1,5-Cyclooctadiene, purity 98%
Results:	1,5-Cyclooctadiene was immediately irritant to the eyes, but produced only a mild conjunctivitis that faded within 24 hours. The main effect was associated with the eyelids, which became red and swollen and exuded a purulent discharge. The blepharitis caused by 1,5-cyclooctadiene took a few days to heal.
Reference:	Brown, V. K. H. and C. G. Hunter (1968). Br. J. Ind. Med., 25:75-76. Brown, V. K. H. et al. (1967). Ann. Occup. Hyg., 10:123-126.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Eye Irritation: None Found.

5.2 Repeated Dose Toxicity

Type:	**2-Week Inhalation and Neurotoxicity Study**
Species/Strain:	Rats/Crl:CD®BR
Sex/Number:	Male/20 per exposure level
Exposure Period:	2 weeks (a total of 9 exposures)
Frequency of Treatment:	6 hours/day
Exposure Levels:	0, 50, 150, 500 ppm
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

Male rats (approximately 8 weeks old, weighing 261-312 g) were exposed, whole-body, for 6 hours/day, for a total of 9 exposures over a 2-week period. Ten rats/group were used for standard toxicological evaluations and 10 rats/group were used for neurotoxicity testing. All rats were weighed and individually observed for clinical signs of toxicity throughout the exposure period. Surviving rats were weighed and individually observed for clinical signs of toxicity throughout the recovery period.

Vapor atmospheres of 1,5-cyclooctadiene were generated by metering the liquid test substance into a heated, Instatherm flask with a syringe infusion pump. Nitrogen was used to carry the test substance vapor from the flask into a glass transfer tube, where it was mixed with filtered, houseline air and fed into the top of the exposure chamber. The concentration of 1,5-cyclooctadiene was controlled by varying the amount of test substance delivered to the flask. The atmospheric concentration of the test substance was determined by gas chromatography at approximately 1-hour intervals during each exposure. Gas samples were drawn by vacuum pump from representative areas of the chamber where rats were exposed. Total chamber airflow, temperature, relative humidity, and oxygen concentration were recorded.

In the standard toxicology group, at the end of the exposure period, and after a 2-week recovery period rats were given clinical and pathological examinations. At each sampling time, after an approximate 16-hour fast, blood was collected and 15 hematologic and 17 clinical chemistry parameters

were measured or calculated. On the day prior to bleeding, an overnight (approximately 16-hour) urine specimen was collected and 9 urine parameters were examined.
Five rats/group were sacrificed and necropsied on the day following the 9th exposure (test day 12) and after 14 days of recovery (test day 25). The liver, kidneys, lungs, testes, and brain were weighed. Each rat was given a complete gross examination and representative samples of approximately 36 tissues were saved for histopathologic evaluation (including prostate, seminal vesicles, testes, and epididymides). All tissues from the 0 and 500 ppm groups sacrificed on test day 12 were microscopically examined. Nose, pharynx/larynx, lungs, liver, and kidneys from the 50 and 150 ppm groups at test day 12 and 0 and 500 ppm groups at test day 25 were microscopically examined. In addition, the kidneys from the 150 ppm group and the pharynx/larynx from the 50 and 150 ppm concentration groups at test day 25 were microscopically examined due to target organ potential.

Rats designated for neurotoxicity evaluation underwent functional observational battery (FOB) assessments (encompassing approximately 36 endpoints) and motor activity (MA) evaluations (encompassing 2 dependent variables) after the 4th and 9th exposures. After approximately 2 weeks of exposure, 6 of 10 neurotoxicity rats/exposure group were sacrificed, examined grossly, and perfused for neuropathology evaluations. Approximately 14 tissue samples from the nervous system and skeletal muscle were saved. Only tissues from the 0 and 500 ppm groups were processed for histopathology and examined. The remaining 4 rats/group were sacrificed with no pathological evaluation.

Body weights, body weight gains, clinical pathology measurements, final body weights, and absolute and relative (to body weight and to brain weight) organ weights were statistically analyzed by a one-way analysis of variance. Pairwise comparisons between test and control groups were made with the Dunnett's test. Pairwise comparisons between test and control groups for body weights and body weight gains were also made with the Least Significant Difference (LSD) test. Increases in the incidence of clinical observations of toxicity were evaluated by the Cochran-Armitage trend test.

The Bartlett's test for homogeneity of variances was performed on clinical pathology and organ weight data, and if significant, the Kruskal-Wallis test was employed and the Mann-Whitney U test was used to compare means from the control group and each of the groups exposed to the test substance.

Descriptive FOB parameters were evaluated by the Cochran Armitage test for trend. If significant, this test was repeated sequentially dropping off the highest dosage level until no further significance was found. If the Cochran-Armitage test for trend was not significant and there was a significant lack of fit, the Fisher's exact test was used with a Bonferroni correction. Forelimb and hindlimb grip strength, and hindlimb foot splay were analyzed as parametric data. Bartlett's test for homogeneity was used to estimate the probability that the groups had different variances. Bartlett's test was not significant, so the data were then analyzed via univariate analysis of variance, with Dunnett's test used to identify which dosage groups, if any, were significantly different from the control group. Separate analyses were performed on the data collected on each test day.

Motor activity data were examined with the Shapiro-Wilk's Test and Levene's Test to detect deviations from a normal distribution and equality of variance, respectively. Non-parametric tests (Kruskal-Wallis followed by Dunn's test and/or Jonckheere) were used because the assumptions of equal variance or normal distribution were violated. For motor activity data, block (10-minute epoch) was an additional repeated measures factor. Contrasts were used to identify which dosage groups were significantly different from the control group on the different test days, and in the case of motor activity, block within test day. Separate statistical analyses were performed on data for male and female rats. Except for the Bartlett's test ($p<0.005$), all significance was judged at $p<0.05$.

GLP: Yes

Test Substance: 1,5-Cyclooctadiene, purity >99%

Results: Mean analytically determined vapor concentrations of 1,5-cyclooctadiene for the 3 test chambers were 52, 150, and 500 ppm. The chamber oxygen concentration measurements were between 20 and 21%, the relative humidity for the chambers was between 20 and 54%, and the chamber temperatures were between 24 and 28°C.

In rats exposed to 500 ppm of 1,5-cyclooctadiene there was an absence of alerting response toward the end of the daily 6-hour exposures. These rats appeared to recover within ½ hour after exposure. These effects were not observed in the other test groups. No other test substance-related changes in clinical signs or body weight parameters were observed.

The FOB evaluation showed an increase in the number of rats found sleeping in the 150 and 500 ppm groups compared to controls after the last exposure, but there were no treatment-related effects in the motor activity evaluation. Since there were no other neurobehavioral findings and no toxicity findings in the 150 ppm group, the sleeping behavior in the 150 ppm group was considered insufficient evidence of an adverse effect.

Clinical laboratory evaluation of the 500 ppm group showed urinary pH decreases at the end of the exposure period, but not after the 2-week recovery period. There were no other toxicologically important changes in urine analysis, hematologic, or blood chemistry evaluations attributable to the test substance.

Histologic effects attributable to the test substance were found in the nose and kidneys of rats in the 500 ppm group. There was a degeneration/necrosis of nasal olfactory epithelium observed immediately after the exposure period and a degeneration/regeneration in this area observed after the 2-week recovery. In addition, there were increased kidney weights in the 500 ppm group immediately after exposure, along with increased hyaline droplets in the kidneys. These effects were reversible after the 2-week recovery period. There were no significant nasal or kidney effects observed in the other test groups, and no other organ weight or histological effects attributable to the test substance were observed in the standard toxicology groups at either evaluation time. The neuropathologic evaluation showed only 1 minor lesion in one 500 ppm group rat, which was not considered to be attributable to exposure to the test substance.

Based on the decreased alerting response observed in rats during exposure at 500 ppm, and on the effects observed in the nose, kidney, and urine in rats at this concentration, the no-observed-effect level (NOEL) in this study was

considered to be 150 ppm.

Reference: DuPont Co. (1996). Unpublished Data, Haskell Laboratory Report No. 351-96, "Two-Week Inhalation Toxicity and Neurotoxicity Studies with 1,5-Cyclooctadiene in Male Rats" (January 16).

Kelly, D. P. et al. (1998). Toxicologist, 42:35 (Abstract 174).

Kelly, D. P. et al. (2001). Drug Chem. Toxicol., 24(3):221-237.

Reliability: High because a scientifically defensible or guideline method was used.

Additional References for Repeated Dose Toxicity: None Found.

5.3 Developmental Toxicity: No Data.

5.4 Reproductive Toxicity: No Data.

5.5 Genetic Toxicity

Type: ***In vitro*** **Bacterial Reverse Mutation Assay**

Tester Strain: *Salmonella typhimurium* TA100, TA1535, TA97a, TA98 *Escherichia coli* WP2*uvr*A (pKM101)

Exogenous Metabolic Activation: With and without Aroclor®-induced rat liver S9

Exposure Concentrations: 0, 10, 50, 100, 500, 1000, 2500, 5000 µg/plate

Method: The procedure used in the test were based on the recommendations of the following guidelines:

EPA Health Effects Testing Guidelines (40 CFR 798.5265 and 798.5100).

OECD Guidelines for Testing of Chemicals Nos. 471 and 472.

EEC Commission Directive 92/69/EEC, Methods B.13 and B.14.

MAFF Japan (59 NohSan No. 4200).

The study consisted of 1 trial with and without activation. Three replicates were plated for each tester strain, test

concentration, and condition. Positive indicators and negative controls were included in all assays. Treatments with activation were conducted by adding 0.1 mL of negative control or test substance solution, 0.5 mL of S9 mix, and 0.1 mL of an overnight culture containing at least $1x10^8$ bacterial to 2 mL of top agar supplemented with 0.05 mM L-histidine and 0.05 mM *d*-biotin for *S. typhimurium* strains or 0.05 mM L-tryptophan for the *E. coli* strain. These components were mixed and poured onto minimal agar plates. Treatments without activation were identical to those with activation with the exception that the S9 mix was replaced with 0.5 mL of sterile phosphate buffered saline. Revertant colonies were counted after the individually labeled plates were incubated at approximately 37°C for about 48 hours. When necessary, plates were refrigerated prior to counting.

Solutions of the test substance were prepared immediately prior to treatment and were presumed to be stable under the conditions of the study. Treatment and control dosing solutions were not analyzed for concentration, uniformity, or stability.

For each tester strain, the average number of revertants and the standard deviation at each concentration with and without S9 activation were calculated.

A test substance was classified as positive when the average number of revertants in any strain at any test substance concentration studied was at least 2 times greater than the average number of revertants in the negative control, and there was a positive dose-response relationship in that same strain. A test substance was classified as negative when either there was no test substance concentrations with an average number of revertants which was at least 2 times greater than the average number of revertants in the negative control, and there was no positive dose-response relationship.

GLP:	Yes
Test Substance:	1,5-Cyclooctadiene, purity > 99%
Results:	Negative
Remarks:	No evidence of mutagenic activity was detected with or without metabolic activation.
Reference:	DuPont Co. (1996). Unpublished Data, Haskell Laboratory Report No. 836-96, "Mutagenicity Testing of 1,5-Cyclooctadiene in the *Salmonella typhimurium* and

Escherichia coli Plate Incorporation Assay" (November 20).

Reliability: High because a scientifically defensible or guideline method was used.

Additional Reference for *In vitro* Bacterial Reverse Mutation Assay:

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Dean, B. J. et al. (1985). Mutat. Res., 153:57-77.

Type:	***In vitro* Clastogenicity Study**
Cell Type:	Human lymphocytes
Exogenous Metabolic Activation:	With and without Aroclor®-induced rat liver S9
Exposure Concentrations:	Trial 1: 0, 0.1, 0.25, 0.5, 0.75, 1.0 mg/L Trial 2: 0, 0.1, 0.25, 0.5, 0.75, 1.0, 2.5, 5.0 mg/L Trial 2 (supplemental harvest): 0.75, 1.0, 2.5, 5.0 mg/L
Method:	The procedure used in the test were based on the recommendations of the following guidelines: EPA Guidelines 40 CFR Part 798, Health Effects Testing Guidelines, 50 FR39443 (1985). EPA Guidelines 40 CFR Part 798, Health Effects Testing Guidelines 52 FR 19079 (1987). OECD Guideline for Testing of Chemicals, No. 473 (adopted 26 May 1983, OECD, Paris). A pair of healthy donors, 1 male and 1 female were used in each experiment. Venous blood was drawn aseptically and cultured replicate cultures were initiated for each test condition, including controls and positive indicators; 1 replicate was from a male donor and the other from a female donor. The cultures were incubated at approximately 37°C. All dilutions of the test substance were prepared in acetone immediately prior to treatment of the cultures. Untreated controls were included in trial 2 and supplemental trial 2. Treatment and control dosing solutions were not analyzed for concentration, uniformity, or stability.

Cytotoxicity Assessment: Studies of cell proliferation, with and without S9 activation, were conducted to assess cytotoxicity. Selection of test concentrations and harvest times for the chromosome aberration trials were based, where appropriate, on the assessment of cell-cycle delay. Evaluation of the mitotic index (MI) was also used in determining cytotoxicity of the test substance.

Approximately 48 hours after culture initiation, the medium was replaced with treatment medium. All test concentrations and solvent controls were evaluated in replicate cultures both with and without activation. After addition of the test or control substance, the cultures were incubated for approximately 3 hours at 37°C. Following treatment, the cultures were rinsed and 5 mL of fresh medium containing 5-bromodeoxuridine were then added. Incubation continued for approximately 23 hours, with Colcemid® present during the final 2 hours to arrest cells in metaphase. Cultures were handled under yellow filtered lights to avoid photolysis of BrdU-substituted DNA.

Cells were harvested, fixed, and dropped onto microscope slides that were then air dried. Sister chromatid differentiation was achieved using a modified fluorescence-plus-Giemsa method. Slides were stained, rinsed, and exposed to 15W UV fluorescent light for approximately 2 hours while immersed in heated phosphate buffer. After rinsing in water, the slides were stained with Giemsa in phosphate buffer and rinsed again. Slides were dried and coverslipped.

Slides from test substance-treated cultures were compared with the solvent controls for evidence of cell-cycle delay. Where possible, 50 metaphase cells were scanned and scored as having gone through one (M1), one-to-two (M1+), two (M2), two-to-three (M2+), or three (M3) DNA replication cycles in the presence of BrdU. Those cells were distinguished by their characteristic chromosome staining, which resulted from differential uptake of BrdU. Cell-cycle delay was judged to be present where the proportion of slower-cycling cells was clearly increased relative to the control, and consequently the average generation time (AGT) was increased.

An appropriate harvest time was chosen for the chromosome aberration trials to allow an estimated 1 to 1.5 cell cycles to

elapse after cell treatment. A second, supplemental harvest was conducted24 hours after the initial harvest for trial 2. This was added to comply with the United Kingdom Environmental Mutagen Society (UKEMS) recommended procedures (1990) for the *in vitro* metaphase chromosome aberration assay, to detect a positive response, which may not be observed at the earlier sampling time.

Chromosome Aberration Trials: Culture initiation, treatments, and cell harvests were conducted as described for the cytotoxicity assessment except that: (1) BrdU was omitted from the medium, (2) test concentrations and harvest times were adjusted, as appropriate, (3) positive indicators were included, and (4) at least 2 independent trials were conducted. Slides were stained with Giemsa. Well spread metaphases were evaluated for structural chromosome aberrations at 1000x magnification. For each trial with and without activation, approximately 100 cells (50 from each replicate; 50 from male, 50 from female) were analyzed whenever possible for each test level, the controls, and the positive indicator. Except for abnormal cells (e.g., damaged cells where the centromere number was difficult to determine with certainty), only cells with 46 centromeres were scored. The aberrations observed were tabulated and categorized as chromatid- or chromosome-type aberrations.

For each trial, the proportion of abnormal cells and the proportion of cells with more than 1 aberration were evaluated with the Fisher's Exact Test to compare each treatment level with the negative (solvent) control, where statistical significance was judged at the 5% level. A Cochran-Armitage test for linear trend (dose-response) was performed where appropriate; significance was judged at the 1% level. Chromatid and isochromatid gaps were excluded from the statistical evaluation.

The test substance was classified as clastogenic (positive) if both of the following effects were reproducible (i.e., evident in 2 or more trials under activated or nonactivated conditions): (1) the test substance produced a statistically significant increase in percent abnormal cells as compared to the negative (solvent) control at 1 or more test concentrations, and (2) there was a statistically significant dose-related increase in percent abnormal cells. The test substance was classified as nonclastogenic (negative) if the following criteria were met: (1) the test substance did not

	produce a statistically significant increase in percent abnormal cells at any concentration tested, and (2) there were no statistically significant dose-related increase in percent abnormal cells.
GLP:	Yes
Test Substance:	1,5-Cyclooctadiene, purity > 99%
Results:	Negative
Remarks:	Toxicity, measured as a >50% reduction in mitotic index, was observed at concentrations of =0.75 mg/L in Trial 1, and =2.5 mg/mL in Trial 2 without metabolic activation. With metabolic activation, toxicity was observed at concentrations of 1.0 mg/mL in Trial 1, and =2.5 mg/mL in Trial 2. In the supplemental trial 2, no substantial cytotoxicity was observed without metabolic activation. With metabolic activation, toxicity was observed at concentrations of =0.75 mg/mL. No statistically significant increases in the percent of abnormal cells were observed at any concentration. No concentration related trends in chromosome aberration induction were observed. The test substance was not clastogenic in this assay. As expected, the positive indicators induced statistically significant increases in the percent of abnormal cells.
Reference:	DuPont Co. (1997). Unpublished Data, Haskell Laboratory Report No. 1997-00114, "1,5-Cyclooctadiene: *In vitro* Evaluation for Chromosome Aberrations in Human Lymphocytes" (June 27).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional Reference for *In vitro* Clastogenicity:

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Dean, B. J. et al. (1985). Mutat. Res., 153:57-77.

Type:	***In vivo* Rat Micronucleus Assay**
Species/Strain:	Rats/Crl:CD®(SD)BR
Sex/Number:	Male/5 or 10
Route of Administration:	Inhalation
Concentrations:	0, 1500 ppm
Method:	The procedure used in the test were based on the

recommendations of the following guidelines:

EPA Guideline 40 CFR 798.5935,

OECD Guidelines for the Testing of Chemicals, No. 474 (adopted 26 May 1983, OECD, Paris), and

Guidelines of the Japanese Ministry of Public Works.

The following guideline exceptions were made: (1) both sexes were not evaluated and (2) bone marrow smears were not prepared at more than 1 time point after the multiple exposure regimen. These exceptions were not considered to affect the quality or integrity of the study.

The animals were 52 or 53 days old and weighed 229.2-246.8 grams at the time of treatment. A group of 5 male rats was exposed to the negative control (air), and another group of 10 male rats was exposed to the test substance. The exposure was for 6 hours/day for 2 consecutive days. Both groups were sacrificed approximately 24 hours after the end of the 2nd exposure. The positive indicator group (5 male rats) was dosed with cyclophosphamide on the day that coincided with the end of the 2nd exposure for the test and negative control groups. The positive indicator rats were sacrificed approximately 24 hours post-dosing. Clinical signs and body weights were recorded.

Vapor atmospheres containing 1500 ppm 1,5-cyclooctadiene were generated by metering the liquid test substance into a heated, Instatherm flask with a syringe infusion pump. Nitrogen was used to carry the test substance vapor from the flask into a glass transfer tube where it was mixed with filtered, houseline air and fed into the top of the exposure chamber. The concentration of 1,5-cyclooctadiene was controlled by varying the amount of test substance delivered to the flask. Rats were exposed whole-body to the test substance in wire-mesh cage modules positioned within the exposure chambers.

The atmospheric concentration of 1,5-cyclooctadiene was determined by gas chromatography at approximately 20 to 50 minute intervals during each exposure. The control level chamber was analyzed at approximately 2.5 to 3.5 hour intervals. Gas samples were drawn by vacuum pump from

representative areas of the chamber where rats were exposed. Total chamber airflow, temperature, relative humidity, and oxygen concentration were recorded.

Immediately after sacrifice, marrow from 1 femur of each rat was aspirated, prepared, and at least 2 slides per animal were prepared, fixed, and stained in acridine orange. Prior to scoring, a coverslip was floated on each slide.

Representative slides from each animal were examined blindly using incident light fluorescence microscopy. Only cells with good morphology and staining were scored. Polychromatic erythrocytes (PCEs; 2000 per animal) were scored for the presence of micronuclei. Cellular inclusions that were irregularly shaped or stained, or out of the focal plane of the cell were considered artifacts. The unit of scoring was the micronucleated cell; PCEs with more than 1 micronucleus were scored as a single micronucleated PCE (MNPCE). Micronucleated NCEs (normochromatic erythrocytes) seen in the optic fields scored to obtain 2000 PCEs were also counted. Additionally, the number of PCEs among 1000 erythrocytes was recorded for each animal.

Data for the proportion of MNPCEs among 2000 PCEs and the proportion of PCEs among 1000 erythrocytes (MNPCE frequency and PCE frequency, respectively) were transformed prior to analysis using the arcsine square root function. Transformed data for PCE and MNPCE frequencies were analyzed separately for normality of distribution and equal variance using the Shapiro-Wilk and Bartlett's tests, respectively. Positive indicator data were not included in evaluating normality of distribution. Results indicated that the transformed values for MNPCE frequency were normally distributed and had equal variance. Therefore, parametric statistics (viz., analysis of variance (ANOVA)) were performed using the transformed data. For analysis of PCE frequency, results indicated that data from the treatment group were normally distributed and had equal variance. Therefore, parametric statistics were performed using the transformed data. For analysis of PCE frequency in the positive indicator group, results indicated that the data had unequal variance. Therefore, non-parametric statistics (viz., Mann-Whitney U test) utilizing non-transformed data were employed. Body weight data were assumed to be normally distributed and were analyzed by ANOVA. Data

	from each group were analyzed separately. All analyses were conducted at a significance level of 5%, except Bartlett's test, which was conducted at a significance level of 0.5%.
GLP:	Yes
Test Substance:	1,5-Cyclooctadiene, purity > 99%
Results:	Negative
Remarks:	The mean concentration (± standard deviation) of 1,5-cyclooctadiene in the exposure chamber over the 2 exposure days was 1500 ± 160 ppm. The temperatures in the 1,5-cyclooctadiene test chamber ranged from 24 to 26°C, relative humidity ranged from 22 to 35%, and the chamber air flow was 35 L/min. During exposure, rats in the 1500 ppm group exhibited depression or absence of an alerting response mainly during the latter half of the daily exposures, and incoordination was evident in 3/10 rats during the 1st exposure. All treatment rats showed incoordination after the 1st exposure, and 2/10 rats exhibited abnormal gait after the 2nd exposure. In addition, irregular respiration was observed in 6/10 rats after the 1st exposure and in 3/10 rats after the 2nd exposure. Nine of 10 rats lost weight after the 1st exposure and all rats lost weight after the 2nd exposure. A statistically significant body weight loss was found in the treatment group on test day 3, prior to sacrifice. No test substance-induced mortality was observed. No statistically significant increase in the frequency of micronucleated PCEs was observed in 1,5-cyclooctadiene-treated rats. A statistically significant depression in the proportion of PCEs among 1000 erythrocytes was observed in the treatment group, indicative of test substance-induced bone marrow toxicity. As expected, a statistically significant increase in MNPCE frequency was found in the cyclophosphamide-treated rats.
Reference:	DuPont Co. (1996). Unpublished Data, Haskell Laboratory Report No. 1027-96 "1,5-Cyclooctadiene: Inhalation Micronucleus Study with Rats" (March 31).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for *In vivo* Genetic Toxicity: None Found.